

We claim:

1. (Amended) A method for identifying a non-metal ion activator of a transition metal-dependent repressor of gene expression in a prokaryote, comprising:

(a) providing recombinant cells comprising a first recombinant DNA segment containing a first promoter operably linked to a first regulatory gene encoding a first repressor native to or functional in a given prokaryote, a second DNA segment containing a second promoter operably linked to a first operator that binds said first repressor and a second regulatory gene encoding a second repressor, and a third recombinant DNA segment comprising a third promoter operably linked to a second operator that binds the second repressor, and a reporter gene;

(b) culturing said recombinant cells in medium substantially free of metal ion activators of said first repressor and which contains a selection agent that directly or indirectly causes a detectable response upon expression or lack of expression of the reporter gene;

(c) adding a non-metal ion test substance to said medium; and

(d) determining whether the response occurs as an indication of whether said test substance activates said first repressor.

2. The method of claim 1 wherein said first regulatory gene encodes a diphtheria tox repressor (DtxR) protein and said first operator binds said DtxR protein.

3. The method of claim 2 wherein said first regulatory gene encodes DtxR and said first operator comprises native tox operator, a functional fragment of said operator or a variant of a DtxR consensus binding sequence.

4. The method of claim 1 wherein said first regulatory gene encodes a diphtheria tox repressor (DtxR) homologue and said first operator binds said DtxR homologue.

5. The method of claim 4, wherein said DtxR homologue is an iron dependent regulator (IdeR) and said first operator binds said IdeR.

6. The method of claim 1, wherein said first repressor encodes ferric uptake regulator (Fur).

7. The method of claim 1 wherein said second regulatory gene encodes TetR and said second operator comprises *tetO*.

8. The method of claim 1 wherein said reporter gene encodes chloramphenicol acetyltransferase and said selection agent is chloramphenicol.

9. The method of claim 1 wherein said medium comprises a chelating agent that binds metal ion activators of said first repressor.

10. The method of claim 9 wherein said chelating agent is 2,2'-dipyridyl.

11. The method of claim 1 wherein said first and second recombinant DNA segments are contained in a first vector and said third recombinant DNA segment is contained in a second vector.

12. The method of claim 11 wherein said second vector is a lambda phage.

13. The method of claim 1 wherein said cells are *E. coli* cells.

14. A method for identifying a non-metal ion activator of a diphtheria tox repressor (DtxR) protein in a prokaryote, comprising:

(a) providing recombinant cells comprising a recombinant vector, wherein said vector comprises a first DNA segment containing a first promoter operably linked to a first regulatory gene encoding a DtxR protein, a second DNA segment comprising a second promoter operably linked to an operator that binds said DtxR protein and a second regulatory gene encoding a tetracycline repressor (TetR), and a third DNA segment comprising a third promoter operably linked to a tetracycline operator (*tetO*) and a reporter gene encoding chloramphenicol acetyltransferase;

(b) culturing said recombinant cells in medium substantially free of metal ion activators of said DtxR protein and which comprises chloramphenicol;

(c) adding a test substance to said medium; and

(d) determining the extent of growth of said cells as an indication of whether said test substance activates said DtxR protein.

15. A method for identifying a non-metal ion activator of a metal-dependent repressor of gene expression in a prokaryote, comprising:

providing a solution containing (a) purified repressor native to or functional in a given prokaryote; (b) a DNA construct comprising in operable association, a promoter, an operator and a reporter gene; (c) a coupled transcriptional and translational system that allows expression of said reporter gene; (d) a chelating agent that binds metal activators of said repressor; and (e) a non-metal test substance to allow a reaction to occur; and

detecting expression or lack of expression of said reporter gene as an indication of whether the test substance activates said repressor.

16. The method of claim 15 wherein the coupled transcriptional and translational system comprises bacterial extract.

17. The method of claim 15 wherein said reporter gene encodes  $\beta$ -galactosidase or luciferase.

18. A composition of matter, comprising: a recombinant vector comprising a first DNA segment containing a first promoter operably linked to a first regulatory gene encoding a first repressor native to or functional in a given prokaryote, and a second DNA segment containing a second promoter operably linked to a first operator that binds said first repressor, and a second regulatory gene encoding a second repressor.

19. The composition of matter of claim 18 wherein said recombinant vector further comprises a third DNA segment

comprising a third promoter operably linked to a second operator that binds the second repressor, and a reporter gene.

20. The composition of matter of claim 18 wherein said recombinant vector is a first recombinant vector and said composition further comprises a second recombinant vector comprising a third DNA segment comprising a third promoter operably linked to a second operator that binds the second repressor, and a reporter gene.

21. The composition of matter of claim 18 which is an *E. coli* cell.

22. The composition of matter of claim 20 which is an *E. coli* cell.

23. A composition of matter comprising: (a) purified repressor protein native to or functional in a given procaryote, a, (b) a DNA construct comprising in operable association, a promoter, an operator that binds said repressor protein and a reporter gene, (c) a transcriptional and translational system that allows expression of said reporter gene and (d) a chelating agent that binds metal ion activators of said repressor protein.

24. The composition of matter of claim 23 further comprising a non-metal ion test substance.

25. The composition of matter of claim 23 wherein said system comprises bacterial extract.